sequence (SEQ. ID NO.: 5). The sequence (SEQ ID NO.: 6)from BAV3 is shown aligned with the sequence from Ad5.

Kindly replace the paragraph beginning at page 44, line 1, with the following

generated by homologous recombination between a transfer plasmid named pTG 14866 (treated with Xbal and calf intestinal phosphatase, CIP) and a plasmid containing a modified version of the hFIX gene (pTG 14869, digested with Sfl). pTG 14866 contains the AdS cis-acting elements plus promoter sequences and the 3' end of the hFIX gene, separated by a unique XbaI site and was constructed as follows. pTG 8343, containing the 5' end of the AdS genome with a deletion in the El region is used as a source for the 5' end of the minimal vector. It is digested with AatII-BglII and overhanging end are made blunt using Klenow enzyme. The Ad5 3' ITR is isolated from plasmid pTG 5670 which contains the ITR, flanked by a PacI site on one end and a multiple cloning site on the other. The ITR is excised as a BgIII fragment, treated with Klenow enzyme and ligated into pTG 8343 prepared as described above. The resulting plasmid, pTG 14138, contains the 5' end from Ad5 from nt 1 to 454, a MCS and the 3' ITR from nt 35828-35938. 5'flanking sequences from the hFIX gene are isolated from pTG 3960 (described in Jallat et al.) as a 1.4 kb fragment by digestion with PstI and HindIII, and introduced in PstI-HindhII digested pTG 14138 by ligation resulting in pTG 14159. Next, 3' flanking sequences from the hFIX gene are isolated from pTG 3960 as a PCR fragment. To this end, primers OTG 2070 (SEQ ID NO.: 1) 5'-AGAGCTTGTATGGTTATGGAGG-3') and OTG 1224 (SEQ ID NO.: 2) (5'-CACGATACTCGATGCAAGAC-3') are used to amplify a 1.6 kb fragment which is

introduced by ligation in pTG 14159 which was linearized with XbaI, and made blunt using Klenow enzyme. This finally resulted in transfer plasmid pTG 14866. Before hFIX sequences were introduced in this transfer plasmid, we removed *5* consecutive PacI sites present in intron D of the hFIX gene in plasmid pTG 3960. This is necessary, because we will use Pad to excise the final minimal vector from the plasmid vector backbone. If Pad sites are present in the minimal vector sequences, this will result in fragmentation of the minimal vector genome. In order to

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Kindly replace the paragraph beginning at page 45, line 9, with the following:

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pTG 14872: In parallel with pTG 14342, a minimal vector containing hFIX as stuffer DNA was constructed. Instead of using transfer plasmid 14866 containing the *5* 'and 3' flanking sequences of the hFIX gene, pTG 14868 was used. It contains part of intron A downstream of the adenoviral 5' hR and encapsidation signal instead of sequences upstream of the hFIX promoter. The construction was started with pTG 14138, which contains the AdS cis-acting sequences separated by a MCS (see above) and which was linearized in the MCS by digestion with HindIII followed by treatment with Klenow. Into this vector was ligated a 1.1 kb SeaI fragment from pTG 3960 containing part of intron A from the hFIX gene. pTG 14160, with the insert in the sense orientation was thus obtained. Next a unique BamHI site in the remainder of the MCS between the AdS packaging signal and the intron A insert was destroyed, while at the same time a NotI site was introduced. This

was done by digesting pTG 14160 with BamHI and ligating oligonucleotide OTG 3497 treated with polynucleotide kinase in the BamHI site. OTG 3497 (SEQ ID NO.: 3) (5'-GATCGCGGCCGC-3') is capable of forming a NotI site flanked by BamHI compatible cohesive ends by auto-hybridization. The resulting plasmid, pTG 14867, is used to introduce the 3' flanking region of the hFIX gene needed for homologous recombination in the next step. The 3' flanking sequence is isolated as an XbaI-KpnI fragment from pTG 14866 and ligated into XbaIKpnI digested pTG 14867. The resulting transfer plasmid pTG 14868 is treated with XhaI and SfI, which allows for the

Kindly replace the paragraph beginning at page 46, line 6, with the following:

pTG 14742: We have chosen to introduce expression cassettes in the unique PmeI site of the empty minimal vector pTG 14872. The PmeI site is located in intron D. In order to facilitate introduction of foreign cassettes, we have sub-cloned a fragment containing the PmeI site in a small vector, flanked by NotI sites. Introduction of the expression cassette in the PmeI site can be done in this vector. After excision of the expression cassette with flanking hFIX sequences by the action of NotI endonuclease, the cassette can be introduced by homologous recombination in the empty minimal vector which has been linearized with PmeI. The first step in the construction of the transfer plasmid consisted in introducing an EcoRI site flanked by NotI sites in phagemid pBluescript II $SK^+$ which was purchased from Stratagene. The

phagemid was digested with PvuII and CIP, thus removing a *445* bp fragment containing the MCS flanked by bacteriophage 13 and T7 promoter sequences. The restriction enzyme recognition sites were introduced by ligation using a polynucleotide kinase treated, self-complementary oligonucleotide (OTG 1 2753 (SEQ ID NO.: 4), 5'-CAGGCGGCCGCGAATTCGCGGCCGCCTG-3'). The resulting plasmid is named pTG 15154 and can be used directly to introduce a hFIX fragment. This was done by cutting pTG 15154 with EcoRI and removing terminal *5'* phosphate groups with CIP followed by ligation involving a 3.0 kb MfeI fragment from pTG 3960 containing the PmeI site. The resulting transfer plasmid pTG 14742 can be used directly for the introduction of foreign DNA sequences.